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EFFECT OF COMBINED INOCULATION WITH CYANBACTERIA AND *Azotobacter* ON WHEAT CROP CULTIVATED IN SANDY SOIL UNDER DIFFERENT NITROGEN LEVELS

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ABSTRACT

To study the effect of combined inoculation with *Azotobacter* and cyanobacteria on wheat production, a field experiment was carried out in the winter season of 2007-2008 using both microorganisms either each separately or in dual inoculation under three levels of nitrogen (zero, 1/2 and full recommended dose of nitrogen) when wheat is cultivated in sandy soil at the farm of the Agricultural Research Station (ARC), Ismailia Governorate. Results indicated that dual inoculation with both cyanobacteria and *Azotobacter*, generally increased the grain and straw yields, NPK uptake by grains and straw, available NPK in soil after wheat harvesting as well as the soil biological activity over the single inoculation of both microorganisms, which surpasses the treatment received 1/2 N dose only . However, in conclusion, attention should be paid to understand the mechanism of dual inoculation with both cyanobacteria and *Azotobacter* that positively affected wheat production and improved biological, physical and chemical characters for sandy soil.
Keywords: *Azotobacter*, cyanobacteria, nitrogen levels, wheat and sandy soils.

INTRODUCTION

Previously, Alexander (1971) reported that azotobacter needs to a simple organic carbon source for its biological activity to fix nitrogen, so it gets into proto- corporation relationship with cyanobacteria formally called blue green algae, especially *Nostoc* and *Anabuena* to take carbohydrates (resulted from photosynthesis process made by cyanobacteria) that lead to increase the amount of fixed nitrogen by both microorganisms. Recently, and about the same relationship Tantawy (2006) proved that dual inoculation with *Azotobacter* and cyanobacteria combined with 1/4 N dose increased significantly the soil biological activity, which leads to the production of plant growth promoting regulator (PGPR) substances and consequently the amount of fixed nitrogen, available NPK in soil and both maize grain and stover yields over the other tested treatments received single inoculation. However, many authors reported that inoculation with *Azotobacter* and/or cyanobacteria are capable of growing and introducing many active substances, which induce the growth and production many crops. Kumar *et al.* (2001) mentioned that *Azotobacter chroococcum* has the ability to be phosphate solubilizing and phytohormone producing when inoculated to wheat. Kennedy, *et al.* (2004) reported that wheat inoculation with non-symbiotic bacterial diazotrophs (including *Azotobacter*) increased the vegetative growth and grain yield. They added that economic and environmental benefits can include the increase of income due to high yields, the reduction of fertilizers costs and emission of the greenhouse gas (N_2O)

with more than 300 times the global warming effect of CO_2. As well as reduced leaching of $NO_3^=$ to ground water. Obtaining maximum benefits on farms from diazotrophic, plant growth promoting biofertilizers will require a systematic strategy designed to fully utilize all these beneficial factors, allowing crop yields to be maintained or even increased, in despite of fertilizers application are reduced. Sergeeva *et al.* (2002) established that *Nostoc muscorum Ag.* liberated into the culture medium auxin-like substances and demonstrated that a number of cyanobacteria produce, accumulate, and liberate 3-indol acetic acid. Inoculation with cyanbacteria (*Anabaena, Nostoc, Calothrix, Aulosira and Cylindrospermum*) genera to rice field soils and urea supplemented plots was investigated by Adhikary (2002) who reported that nitrogenase activity of the soils inoculated with cyanobacteria was higher than the control and N-fertilizer supplemented plots. Most of the inoculated species competed successfully with the indigenous flora and established in the fields contributing higher amount of fixed N to the soils and an increase of grain yield by over 25 % was obtained in the algalized plots. Also, Mishra and Pabbi (2004) reported that cyanobacteria offer an economically attractive and ecologically sound alternative to chemical fertilizers for realizing the ultimate goal of increased productivity, especially in rice cultivation. In a wetland rice ecosystem, nitrogen fixation by free living cyanobacteria also significantly supplements soil with nitrogen. In very recent reports, Ahmad *et al.* (2008) tested some microbial isolates and found that more than (80 %) of *Azotobacter* isolates produce IAA, whereas (74.47 %) are able to solubilize of phosphate and all the tested isolates produce ammonia.

This work is designed to study the effect of dual inoculation with cyanobacteria and *Azotobacter* either each alone or both in combination on wheat yield, NPK uptake for wheat grain and straw yields as well as biological activity, physical and chemical characters of sandy soil.

MATERIALS AND METHODS

A field experiment was carried out at the experimental farm of Agricultural Research Station, Ismailia Governorate, Agric Res. Center (ARC) Egypt, during the winter season of 2007-2008 under sprinkler irrigation system to study the effect of *Azotobacter*, *cyanobacteria* inoculation each individually and/or both in combination under three nitrogen levels (full N recommended dose, 1/2 full N recommended dose and zero N) on wheat plant. Some soil biological, physical and chemical properties were determined according to the methods described by (Black 1965) and are shown in (Table 1).

The plot area was 17.5 m^2 (3 x 3.5 m), and the experiment was designed in a split-plot design with three replicates, where the bio-fertilizers (inoculation with azotobacter and cyanobacteria) are allocated in the sub plots

Table (1): Chemical characteristics of the studied soil (0-30 cm)

Sample	EC dS/m	pH	Soluble ions (meq/l)								SAR
			Ca^{++}	Mg^{++}	Na^{+}	K^{+}	$CO3^{=}$	$HCO3^{-}$	Cl^{-}	$SO4^{=}$	
Soil	0.37	7.54	1.07	1.82	0.78	0.18	–	1.53	0.96	1.36	0.64

Some physical characteristics of the studied soil (0-30 cm).

Particle size distribution %					Organic matter %	$CaCO_3$ %
Coarse sand	Fine sand	Silt	Clay	Texture class		
31.82	61.61	1.22	5.35	Sandy	0.52	1.42

Initial total count, total nitrogen fixers, *Azotobacter*, N-ase and DHA before planted of the studied soil.

Total count CFU g $soil^{-1}$ $x10^{6}$	Total nitrogen fixers CFU g $Soil^{-1}$ x 10^{4}	*Azotobacter* CFU g $soil^{-1}$ $x10^{2}$	N-ase (nmole C_2H_2 $gdwt^{-1}h^{-1}$)	DHA μLH_2 100g $soil^{-1}h^{-1}$
12	10	6	0.36	420

Nitrogen fertilizers were randomly distributed in the main plots as follows:

I) Full dose recommended (80 kg N/fed).

II) Half dose recommended (40 kg N/fed).

III) Zero N

The recommended doses of phosphorus and potassium fertilizers were added uniformly at the rates of 15 kg P_2O_5 /fed as super phosphate (15.5% P_2O_5) and 48 kg K_2O/fed as potassium sulphate (48% K_2O) before cultivation. Nitrogen was added in the form of ammonium sulphate (20.5 % N) according to the applied treatments into two equal split doses (after 30 and 60 days from cultivation).

Bacterial preparation, inoculation and count methods

Seedlings (15 days after planting) were inoculated during irrigation by culture broth (24 hours prepared) of *Azotobacter chroococcum* containing 10^{8} cell ml^{-1}, and re-inoculated after two weeks later. *Azotobacter* used isolated from soil by the Depat. of Agric. Microbiol. Soils, Water, and Environ. Res. Inst. ARC, Giza, Egypt on the medium of Hegazy and Neimela (1976), growing and maintenance were done by using the same medium. Total count of *Azotobacter* was completed by using the most proble number technique (MPN) using also the same medium., while cyanobacteria were inoculated to wheat using the soil based inoculum (10^{12} cfu g $soil^{-1}$) prepared as described by Venkataraman (1972). The cyanobacteria inoculum is composed of a mixture of individual strains namly *Nostoc muscorum, Nostoc calciccla,Anabaena oryzae* and *Clyndrospermum muscicola*. Total count of bacteria enumerated in soil tested after 45 days from seedlings inoculation and at harvest. For counting total bacteria and nitrogen fixing bacteria the dilution plate method was used on the media of Bridson (1978) and Watanabe and Barraquie (1979), respectively. Cyanobacteria count in soil was carried out by the method described by Allen and Stanier (1968), beside count of *Azotobacter* that mentioned previously.

Straw and grain samples were also oven dried ground and digested according to Thomas *et al.* (1967) then subjected to the determination of NPK contents as described by Van Schouwenburg (1968). Available nutrients in the soil after wheat harvesting were extracted as described by Jackson (1973), i.e. nitrogen by 2N potassium chloride, Phosphorus by 0.5M sodium bicarbonate and potassium by 1N ammonium acetate. Organic matter was determined using the modified Walkly's method, Jackson (1973). The mean weight diameter (MWD) was originally proposed as an index for describing the dry aggregate distribution of a soil by Kemper and Rosenau (1986).

All obtained data were subjected to statistical analysis according to Snedecor and Cochran (1989), where mean values were compared using L.S.D at 5% level.

RESULTS AND DISCUSSION

Wheat grain yield components:

Results in Table (2) show the effect of the bacterial inoculation on wheat yield and its components. Results showed that the inoculation with a composite of *Azotobacter* and cyanobacteria plus 1/2 N dose attained the superior effect on all the examined parameters compared to the single inoculation, the highest mean values of total yield, grain and straw yields were 5200, 2590 and 2610 kg fed^{-1}, respectively, the single inoculation, which came in the second rank in case of cyanobacterial inoculation plus 1/2 N dose reported a good effect on grain yield, and gave the highest values of, 1960.00 kg fed^{-1}, while *Azotobacter* plus 1/2 N dose showed a positive effect on total yield, straw yield and 1000-grain weight. These results are in agreement with those obtained by Kenndy *et al.* (2004), who decided that a range of diazotrophic plant growth-promoting rhizobacteria (including *Azotobacter*) participate in interactions with crop plants (e.g. rice wheat, maize, sugarcane and cotton), significantly increased their vegetative growth and grain yield. This result may due to the nitrogen fixation and plant growth promoter bacteria that increased in presence of *Azotobacter* and cyanobacteria together because of the cooperation relation between them mentioned by Alexander (1971) and consequently enhanced the plant growth parameters.

Table (2): Effect of cyanobacteria and *Azotobacter* inoculation on wheat yield components cultivated in sandy soil

Nitrogen levels	Inoculation	Total yield Kg/fed	Grain yield Kg/fed	Straw yield Kg/fed	1000 grain weight (g)
Zero N	Cyanobacteria	2066.67	1102.50	964.17	33.40
½ full		3666.67	1960.00	1706.67	33.27
full		2666.67	1680.00	986.67	37.10
Zero N	*Azotobacter*	2333.33	910.00	1423.33	34.33
½ full		4133.33	1785.00	2348.33	37.23
full		2933.33	1400.00	1733.33	32.97
Zero N	Cyano + *Azotobacter*	2073.33	1120.00	953.33	36.07
½ full		5200.00	2590.00	2610.00	38.17
full		3466.67	1908.33	1558.33	38.37
L.S.D at 0.05 N x Inoculation		n.s	n.s	n.s	n.s

That relationship emphasized by Adhikary (2002) who reported that most of the inoculated cyanobacterial species competed successfully with the indigenous flora (including *Azotobacter*) and established in the fields contributing higher amount of fixed N to the soil. Increase of grain yield by over 25 % was obtained in algalized plots higher than the control and N-fertilizer supplemented plots.

NPK uptake wheat grain and straw

Data in Table (3) revealed that the superior of dual inoculation (cyanobacteria and *Azotobacter*) was continuous especially when applied in addition to 1/2 N dose. Inoculation with dual inoculation increased available NPK in grains over the single inoculation. The most affected parameter according dual treatment was available nitrogen uptake by grains, which recorded 104.64 kg fed^{-1}. The same treatment was also proceeding in available phosphate and potassium uptake by straw with significant in phosphate uptake compared to single inoculation. Single inoculation with *Azotobacter* plus 1/2 N dose was proceeding only with nitrogen uptake by straw followed by cyanobacterial inoculation plus 1/2 N dose and recorded the values of 19.02 and 18.77 kg fed^{-1}, respectively. These results are confirmed by those obtained due to Hanna *et al.* (2004) who found that inoculation with cyanobacteria increased significantly the nitrogen, phosphorus and potassium contents of wheat grain and straw. Also, Kumar *et al.* (2001) mentioned that *Azotobacter chroococcum* has the ability to fix nitrogen, Phosphate solubilizing and phytohormone producing when inoculated to wheat.

Table (3): Effect of cyanobacteria and *Azotobacter* inoculation on NPK-uptake (Kg fed^{-1}) in wheat grains and straw

Nitrogen levels	Inoculation	Macronutrients uptake (Kg fed^{-1}) by grain			Macronutrients uptake (Kg fed^{-1}) by straw		
		N	P	K	N	P	K
Zero N	Cyanobacteria	39.69	7.17	9.04	5.98	1.74	1.35
½ full		59.39	10.39	15.68	18.77	4.78	5.63
full		55.61	13.61	13.94	7.10	6.22	5.53
Zero N	*Azotobacter*	33.49	6.28	8.37	10.39	2.42	5.69
½ full		73.19	11.60	15.17	19.02	5.64	13.86
full		50.82	9.66	12.18	14.04	4.16	5.20
Zero N	Cyano + *Azotobacter*	49.50	8.40	9.52	8.01	13.92	9.15
½ full		104.64	15.28	23.05	14.09	22.45	23.75
full		85.88	12.79	15.84	13.40	4.99	7.32
L.S.D at 0.05	N x Inoculation	n.s	n.s	n.s	n.s	8.52	n.s

Available NPK, OM and MWD in soil:

Data in Table (4) show available NPK, organic matter and the soil Mean weight diameter (MWD) for the soil remained after wheat harvesting as affected by *Azotobacter* and/or cyanobacterial inoculation under different nitrogen levels. Data revealed that three of the tested treatments were superior in available N with the same value of 140 ppm. These treatments were dual inoculation plus 1/2 N dose, *Azotobacter* plus 1/2 N dose and

Azotobacter with zero nitrogen. In case of available P, the highest values were 8.57 and 8.0 ppm due to dual inoculation plus zero N and *Azotobacter* plus 1/2 nitrogen dose, respectively. Inoculation with *Azotobacter* plus 1/2 N dose was proceeding in organic matter (O.M) recording the highest percentage of 0.33 that obtained from five treatments (dual inoculation plus 1/2 N dose, *Azotobacter* with no N and cyanobacteria with both of the half and full N dose). Finally, the treatment of dual inoculation plus full N dose showed the highest value (0.87 mm) for the soil MWD followed by cyanobacteria plus 1/2 and full N dose (0.78 mm) with no significant difference between them. These results could be explained by that both *Azotobacter* and cyanobacteria are known to excrete extra-cellular compounds to soil, these compounds hold or glue soil particles together in the form of micro-aggregates and hence improve nutrients availability in soil (Mandal *et al.* 1999). Also, in long- term studies to evaluate cyanobacteria effect on soil fertility during periods of soil, Pankratova (2006) reported that cyanobacteria contribute to the nitrogen pool of soils that reached 30 kg/ha, and the concept of transforming the organic matter of cyanobacteria in soil and its movement along atrophic chains of the biological cycle has been developed.

Table (4): Effect of cyanobacteria and *Azotobacter* inoculation on soil available NPK (ppm), O.M and MWD

Nitrogen levels	Inoculation	Available NPK (ppm)			O.M %*	MWD**
		N	P	K		mm
Zero N	Cyanobacteria	116.67	6.30	88.40	0.29	0.61
½ full		112.00	5.47	83.20	0.30	0.78
full		121.33	6.67	91.00	0.30	0.75
Zero N	*Azotobacter*	140.00	5.83	75.40	0.30	0.59
½ full		140.00	8.00	93.60	0.33	0.65
full		116.67	6.20	96.20	0.19	0.64
Zero N	Cyano + *Azotobacter*	126.00	8.57	80.60	0.27	0.69
½ full		140.00	5.13	72.80	0.30	0.53
full		112.00	5.47	75.40	0.27	0.87
L.S.D at 0.05 N x Inoculation		n.s	n.s	n.s	n.s	n.s

* Organic matter ** Mean weight diameter

Soil nitrogenase, dehydrogenase and total bacterial count as affected by cyanobacteria and *Azotobacter* inoculation under different nitrogen levels:

Table (5) shows the bacterial count in tested soil at 45 days and after harvest. Due to total bacteria, both total nitrogen fixers bacteria and *Azotobacter* counts results indicated that dual inoculation increased significantly their counts over those obtained by individual inoculation. The increases were noticed mainly when inoculation was carried out under the effect of 1/2 N dose. These increases were also similar to those obtained by the treatments received no and full of N dose.

Data in Table (6) indicate the dehydrogenase (DHA) and nitrogenase activities measured in wheat rhizosphere area that go side by side in both enzymes except for little differences. Results showed that both nitrogenase and dehydrogenase recorded their highest values with the treatments received 1/2 N dose combined with inoculation of dual *Azotobacter* and cyanobacteria followed by *Azotobacter* inoculation and at last cyanobacteria. Increasing N-level to the full N dose affected these parameters adversely and became worse. These results were in agreement with those obtained by Abd El- Rassoul *et al.*(2004) in wheat, El-Zeky *et al.*(2005) in rice who found that inoculation with *Azotobacter* combined with low level of nitrogen (1/2 N dose) increased significantly both N_2-ase and dehydrogenase activites over the control as a result of microorganisms count increasing. El-Mohandes (2000) explained that high level of N-fertilizer caused an opposite effect on nitrogen fixation as a result of N_2-ase activity inhibition. Also, dehydrogenase activity increased with bacterial inoculation and this was in agreement with Seagnozzi *et al.* (1995) who reported that there is a positive significant relationship between (DHA) activity and microbial count in soil.

Table (5): Effect of cyanobacteria and *Azotobacter* inoculation on total bacteria count, total nitrogen fixer and *Azotobacter* at 45 days and harvest of the studied soil

Nitrogen levels	Inoculation	At 45 days (CFU g soil^{-1})			At harvest (CFU g soil^{-1})		
		Total count x10^6	total nitrogen fixers x10^4	*Azotobacter*. x10^2	Total count x10^6	total nitrogen fixers x10^4	*Azotobacter*. x10^2
Zero N	Cyanobacteria	34.00	41.00	23.00	7.33	7.00	8.00
½ full		66.00	46.00	30.00	22.33	20.33	4.33
full		20.00	22.67	25.00	12.33	7.00	11.33
Zero N	*Azotobacter*	45.00	40.00	210.00	7.00	7.33	170.00
½ full		92.00	51.00	335.00	24.00	27.33	240.00
full		50.00	25.00	190.00	14.33	12.00	170.00
Zero N	Cyano + *Azotobacter*	39.00	46.33	210.00	10.33	12.00	140.00
½ full		105.00	61.00	420.00	49.33	39.00	260.00
full		41.00	12.00	250.00	10.33	19.33	180.00
L.S.D at 0.05	N x Inoculation.	9.63	7.26	88.61	6.96	3.66	34.86

Table (6): Effect of cyanobacteria and *Azotobacter* inoculation on nitrogenase (N-ase) and dehydrogenase (DHA) activities at harvest of the studied soil

Nitrogen levels	Inoculation	N-ase nmolC_2H_2 g d.wt^{-1}h^{-1}	DHA µL H_2 100g soil^{-1} h^{-1}
Zero N	Cyanobacteria	0.77	1200
½ full		1.78	1590
full		0.85	1.24
Zero N	*Azotobacter*	1.23	1160
½ full		2.83	1950
full		1.30	1180
Zero N	Cyano + *Azotobacter*	0.74	1130
½ full		3.23	2.11
full		0.98	980
L.S.D at 0.05	N x Inoculation	0.22	170

Conclusion

In the present study, it could be concluded that dual inoculation with *Azotobacter* and cyanobacteria can save approximately 50 % of the nitrogen amount required for wheat crop rather than the improvement released to the biological, physical and chemical properties of the poor sandy soil. So much attention and more studies should be done to establish this eco-friendly technology towards other cereal crops rather than wheat.

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اثر التلقيح المشترك بالسيانوباكتريا والازوتوباكتر على محصول القمح المزروع فى الاراضى الرملية تحت مستويات نتروجين مختلفة

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الاراضى الرملية الفقيرة البعيدة عن خصوبة الدلتا فى امس الحاجة لزيادة مولدات الخصوبة وعلى راسها التلقيح البكتيرى ، وفى تجربة حقلية فى اراضى محطة البحوث الزراعية فى محافظة الاسماعيلية تم اختبار نوعين من البكتريا لهما القدرة على التعاون معا لزيادة نواتج كلا منهما من تثبيت النيتروجين والمواد المنشطة لنمو النباتات والتى لها القدرة على زيادة محصول القمح لما له من اهمية اقتصادية وبالتالى زيادة خصوبة التربة لبقاء هذة المواد فى التربة بعد الحصاد.

اهم نتائج هذة التجربة يمكن تلخيصها فيما يلى:

١- التلقيح بالازوتوباكتر والسيانوباكتريا معا ادى الى زيادة فى القياسات الحيوية لميكروبات التربة سواء فى اعدادها او نشاطها الانزيمى مقارنة بالمعاملة بكلا منهما منفردا.
٢- التلقيح بالازوتوباكتر والسيانوباكتريا مجتمعين ادى الى زيادة فى كلا من محصول الحبوب والقش ومحتوى الحبوب والقش من عناصر النيتروجين والفسفور والبوتاسيوم ، وايضا ادى الى زيادة قياسات التربة من هذة العناصر بعد الحصاد وكذلك قياسات المادة العضوية فى التربة.
٣- تم تقليل كمية النيتروجين المستخدمة فكانت افضل النتائج مع التلقيح بالازوتوباكتر والسيانوباكتريا مجتمعين او منفردين مع نصف كمية النيتروجين الموصى بها بالمقارنة بكمية النيتروجين الموصى بها كاملة.
٤-النتائج تحفز على الاهتمام بتلقيح الازوتوباكتر مع السيانوباكتريا فى المحاصيل النجيلية للاستفادة من علاقتهما الايجابية ونشاطهما البكتيرى معا للوصول لمعدلات عالية من انتاجية هذة المحاصيل وزيادة خصوبة الاراضى الرملية الفقيرة.